

The Role of Impaired Epidermal Barrier Function in Atopic Dermatitis

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ABSTRACT Atopic dermatitis (AD) is a chronic, inflammatory, pruritic skin disease with increasing prevalence. The etiopathogenesis of atopic dermatitis is multifactorial and involves a complex interplay of environmental and genetic factors that induce derangements in the structure and function of the epidermal barrier and immune system. Due to great heterogeneity of etiopathogenesis, there is also great variability of clinical presentation, and diagnosis can sometimes be challenging and difficult. Diagnosis mostly relies on clinical features and laboratory tests, but morphology alone cannot reliably establish the diagnosis, so the spectrum of features associated with AD must be considered. Traditionally, patients with AD have been separated into two different subgroups, i.e. intrinsic and extrinsic. Today, most of authors prefer the outside to inside and back to outside hypothesis, suggesting that the primary disorder lies in epidermal structure and function, resulting in inflammation and immunological downstream activation which further provokes secondary barrier abnormalities. In this review, we discuss the structure and function of the epidermal barrier and the role of impaired barrier function in etiopathogenesis of atopic dermatitis.

KEY WORDS: atopic dermatitis, skin barrier, atopic barrier, filaggrin, ceramides, *Staphylococcus aureus*, natural moisturizing factors

INTRODUCTION

Atopic dermatitis (AD) is a chronic, inflammatory, pruritic skin disease with increasing prevalence (affecting 15-30% of children and 2-10% of adults) (1-4). It is considered to be the first step in the "atopic march" that can progress to asthma (AA) and allergic rhinitis (AR) (5). Etiopathogenesis is multifactorial and involves a complex interplay of environmental and genetic factors that induce derangements in the structure and function of the epidermal barrier and immune system (6). Due to great heterogeneity of etiopathogenesis, there is also great variability of

clinical presentation and diagnosis can sometimes be challenging and difficult (7). Diagnosis mostly relies on clinical features and laboratory tests, but morphology alone cannot reliably establish the diagnosis, and the spectrum of features associated with AD must be considered. Several sets of diagnostic criteria for AD have been proposed and validated; most clinician traditionally use Hanifin and Rajka's criteria, but full agreement amongst clinicians and uniformity of criteria are still lacking (8,9). Traditionally, patients with AD have been separated into two different



subgroups, i.e. intrinsic and extrinsic. The extrinsic subtype presents with allergic sensitization to an external antigen with subsequent allergen-specific immunoglobulin E (IgE) production; the intrinsic variant is found in patients with all clinical features of AD but no detectable allergen-specific IgE. These subtypes may actually represent different stages of evolution based on the relative degree of sensitization. AD in infancy is thought to begin as "intrinsic"/non-allergic dermatitis, and over time it progresses to "true" atopy in the majority of cases *via* allergen exposure through what is being increasingly widely recognized as a primarily defective epidermal barrier function (6,10). Etiopathogenesis of AD is extremely complex, and several hypotheses have been advanced (6). Traditionally, it was thought that the primary pathogenic mechanism of AD was initiated by immune dysfunction leading to a T helper 2 (Th2) cytokine imbalance, increased inflammation, and secondary disruption of the epidermal barrier, usually referred as the inside to outside hypothesis (11). Recently, after very important findings on filaggrin (FLG) and its enormous impact on the structure and the function of the epidermal barrier, there is new emerging evidence suggesting that a primary defect in the stratum corneum (SC) plays a major role in driving the pathogenesis of AD, leading to sustained cytokine release, recruitment of pro-inflammatory molecules, and stimulation of a Th2 response. This is usually called the outside to inside hypothesis (11-13). Today, most authors prefer the outside to inside and back to outside hypothesis, suggesting that the primary disorder lies in epidermal structure and function, resulting in inflammation and immunological downstream activation which further provokes secondary barrier abnormalities (11,12).

EPIDERMAL BARRIER

The epidermal barrier (EB) is the first line of defense against the environmental influences and prevents water loss and preserves electrolyte balance. The epidermis generates a set of protective/defensive functions mediated by its unique differentiation end product, the stratum corneum (SC) (11). The permeability barrier resides in the SC, which is a multilayered tissue composed of flattened anucleate corneocytes surrounded by multiple lamellar sheets enriched in ceramides (CER), cholesterol (CHOL) and free fatty acids (FFA), surrounded by a cell envelope composed of cross-linked proteins, the corneal envelope (14,15). Nucleated cells with cytoskeleton and tight and gap junctions contribute to the physical barrier. The permeability barrier retards transcutaneous water loss, microbial invasion, and entry of allergens and irritants. Another very important function is providing mechanical support (14).

The chemical barrier is formed by lipids, "acid mantle", antimicrobial peptides secreted by keratinocytes (KC), and the FLG protein that aggregates keratin filaments and produces natural moisturizing factors (NMF). The so called "acid mantle" is provided by free fatty acids (FFA), lactic acid from sweat, and urocanic acid, a degradation product of FLG (14). These components act together in ensuring normal keratinization and lipid synthesis, providing antimicrobial protection, and proper hydration of the skin (14). Hydrophobic lipids within the extracellular domain inhibit the outward movement of the water. Lipids are delivered, like their precursors, through secretion of the epidermal lamellar body (LB). As the SC is formed, these bodies deliver lipid constituents, lipid precursors, and enzymes required to generate CER and FFAs required for their organization into mature membrane structures (15). Additionally, proteases and their inhibitors derive from LB and orchestrate the orderly digestion of corneodesmosomes, allowing corneocytes to shed invisibly at the skin surface (15,16). The immunological barrier is closely linked to innate immunity of the skin. This results from complex interaction of the physical barrier, several immunologically active cell types, action of antimicrobial peptides, cytokines and encoded proteins called pattern recognition receptors (PRR) (14,17). Cells involved in innate immunity are KCs, dendritic cells, Langerhans cells, neutrophils, and natural killer cells (17,18). Immune response and inflammation will be discussed further in the text.

BROAD BARRIER FAILURE IN ATOPIC DERMATITIS IS A DRIVER FOR CHRONICITY OF DISEASE

Dysfunction of the skin barrier in AD is the result of a complex interplay between the patient's genotype, environmental factors, and inflammation activity (11,12,19,20). Barrier abnormalities can be roughly characterized into inherited barrier abnormalities, the group of exogenous and endogenous stressors with further aggravation of barrier function, and impaired antimicrobial defense with further compromised barrier function (12). There is a complex interaction between genetic influences and exogenous and endogenous factors.

Recently, the focus has been on the role of genetic abnormalities which lead to key structural defects in EB, since 80-90% of all AD have a genetic background. The genes affected in AD include those encoding the FLG protein, serin proteases (SP), and protease inhibitors (12,14). Although mutations in FLG are best known, mutations in other members of the fused S-100 family of proteins (hornerin and filaggrin 2, the

cornified envelope precursor (i.e. SPRR3), mattrin, which is encoded by TMEM79 and regulates the assembly of lamellar bodies, SPINK5, which encodes the serine protease inhibitor lymphoepithelial Kazal-type trypsin inhibitor type 1, and the fatty acid transport protein 4 have all been linked to AD (21). These abnormalities often only predispose to AD, and additional acquired stressors that further compromise barrier function, such as psychological stress, low ambient humidity, or high-pH surfactants are often required to trigger the disease (21). Th2 cytokines can also compromise barrier function by downregulating expression of multiple epidermal structural proteins, lipid synthetic enzymes, and antimicrobial peptides. All of these inherited and acquired abnormalities converge on the lamellar body secretory system, producing abnormalities in lipid composition, secretion, and/or extracellular lamellar membrane organization, as well as antimicrobial defense (21).

The mutation of the *FLG* gene (*FLG mut*) has the greatest influence, and today the majority of authors put it at the center of the etiopathogenesis. Filaggrin (*FLG*) was named by the late Peter Steinert, and its gene, among other proteins involved in terminal differentiation, is located on chromosome 1q21, called the epidermal differentiation complex (13). The *FLG* gene (*FLG*) encodes for important multifunctional structural and functional protein of the epidermis. The name "filaggrin" is derived from the term "filament-aggregating protein". Loss-of-function mutations, in one or both alleles, result in reduced or completely absent levels of epidermal *FLG* (22,23). Loss of function mutations in *FLG* are the major risk factor for developing AD (24). Not all AD can be explained by *FLG* mutations however, since only 40% of patients with AD have this mutation, and not all individuals with *FLG mut* will develop AD (24-26). *FLG mut* alone does not suffice, and this fact is clearly shown in ichthyosis vulgaris where *FLG mut* is found in one or both alleles, but inflammation does not occur (12,27). Recent findings report a reduced skin barrier function irrespective of *FLG* genotype, suggesting the importance of additional factors for an impaired skin barrier in AD beside the impact of *FLG* mutation (28,29). About 10% of the general population in Northern Europe are heterozygous mutation carriers, while 0.1% are homozygous (12,30). Data for Southern Europe are scarce, but indicate that these mutations are rare or even absent (31-34). However, prevalence of *FLG mut* is much higher in patients with AD, and prevalence of this mutation is 25-50% in patients with AD in Northern Europe (24). Some authors view the north-to-south gradient in observed prevalence of *FLG mut* as evolutionary, speculating that these mutations have

evolved due to low exposure to the sun and that this might be closely related to vitamin D in lightly pigmented Northerners. It is also known that NMF is responsible for UV protection. Another hypothesis and potential explanation is increased penetration of microorganisms as an *in vivo* mechanism of vaccination (13,35-37).

FLG loss-of-function mutations show population specificity (34). Since the discovery of the first two loss-of-function mutations (R501X and 2282del4) in 2006, these two mutations, along with the less prevalent S3247X and R2447X, have been studied and are present in 7-10% of the Caucasian population of Europe (24,35,41). More than 20 other rare or family-specific mutations have been discovered in white European ancestry (35). The population specificity indicates that they have arisen after one population has become split away from another (35). The Asian population has their own mutation spectra (35,38,39). The prevalence of *FLG* null-mutations varies across Europe, but R501X and 2282del4 are the two most common mutations and they have consistently shown significant association with AD across the continent, with the exception of the Italian population (32,33,35,40). Full sequencing of *FLG* in the Italian population identified only 3 additional mutations and no association with AD (32,33).

In those patients in whom AD is linked to *FLG* mutation, there is a genotype-phenotype correlation (42-44). Prevalent and low-frequency null-mutation in *FLG* is associated with early onset and persistent atopic eczema (42). *FLG* haploinsufficiency is highly penetrant and is associated with increased severity of eczema (43). The phenotype of early onset (before 2 years of age), persistent, and severe eczema has shown the strongest and most significant statistical association with the combined *FLG* null phenotype, with an odds ratio of up to 7.7 (44-46). However, the milder phenotype has also shown a statistically significant association (44,47). Beside loss-of-function mutation of *FLG*, there is another important genetic influence on *FLG* levels (12). The *FLG* gene is a repetitive gene and shows intragenic copy number variations (CNV), with the number of repeats between 10 to 12 (48). All reported *FLG* null-mutations are nonsense or frameshift mutations, resulting in truncation of the profilaggrin molecule. *FLG* encodes for profilaggrin (Pro*FLG*), polymers released from keratohyalin granules of stratum granulosum and proteolytically cleaved into 10-12 identical *FLG* monomers by enzymes. The liberated *FLG* monomers aggregate keratin filaments into tight bundles, resulting in collapse and flattening of corneocytes. *FLG* and other epidermal differentiation-linked proteins are crosslinked

into the cornified envelope by transglutaminases. Keratin 1 and 10 associate with FLG and its degradation products and constitute the bulk of cytosol of the anucleate corneocytes (KC) (12).

Besides structural function and mechanical strength, FLG has another very important function. As KCs move apically, FLG detaches from the cornified envelope, followed by degradation into constituent aminoacids, glutamine, arginine, and histidine and into their deiminated metabolites (12,49-51). Histidine is a substrate for histidase which generates trans-urocanic acid (tUCA), which is a major ultraviolet B (UVB)-absorbing epidermal chromophore that also contributes to the acid mantle of the skin (49). Glutamine is converted into pyrrolidone-5-carboxylic acid (PCA), a major component of "natural moisturizing factors" (NMF) of the SC and therefore a potent humectant, accounting for much water retention in the SC (49-51).

Another function of NMF is acidification, or, more precisely, maintaining proper pH values of the skin. Acidification is very closely connected to the antimicrobial function of the barrier as well as to proteolytic activity and release of proinflammatory cytokines (12,52). A loss of function mutation in *FLG* is strongly associated with an elevated pH of the SC surface, which might negatively influence SC lipid production, activity of various proteases involved in inflammatory processes, and desquamation and antimicrobial defense (12). Therefore, FLG deficiency affects epidermal protein expression and organization (12,53).

There is also evidence of environmental and inflammation-driven reductions in levels of epidermal FLG, and not only due to genetic reasons (12). It is known that monomeric FLG is hydrolyzed in a humidity-sensitive fashion as environmental humidity declines, following a rapid shift from a humid to a dry environment (54). Children with AD linked to *FLG* *mut* more frequently have dermatitis on air-exposed areas when compared to the wild type (55). UVB radiation is another climatic and environmental influence, single dose of UVB exposure leads to downregulation of *FLG* expression (12,56). Beside climatic influence, exposure to water and irritants also reduces FLG levels (57). Mechanical disturbance of the barrier, such as scratching as a result of intense pruritus in AD, also downregulates FLG expression. Moreover, further barrier disruption in the chronic stages of AD through mechanical scratching not only perpetuates, but alters the response to a mostly Th1 type (12). Topical therapy can also have an impact on the expression of epidermal FLG. It is interesting that prolonged use of topical corticosteroids, but not topical tacrolimus, reduces epidermal FLG (21,58,59).

Other genes can be affected and have important influence on epidermal barrier, such as genes that upregulate serin proteases (SPs). Genetic defects due to gain of function mutations in the kalikrein 7 gene encoding serin proteases up-regulate serin proteases (SPs) (14). Increased activity of SPs degrades desmosomes, lipid-processing enzymes which results in decreased production of ceramides (12). It also activates the protease activator type 2 receptor (PAR-2) by direct cleavage and inducing its signal cascade, resulting in downregulation of lamellar body secretion and increased production of interleukin 1 alpha (IL-1 α) and interleukin 1 beta (IL-1 β) from corneocytes leading to inflammation (60,61). Protease inhibitors (PIs) are another important part of the balance due to the fact that these inhibitors inhibit the function of proteases (14). Cystatin A is a cystein PI secreted by sweat glands to inhibit the degradation by exogenous (microbial) proteases. Gene mutations for this PI has been found in patients with AD (14,19,60,62). The best example of impaired protease inhibitor function is in Netherthson syndrome, which is characterized with severe eczema, mucosal atopy, and severe food allergies. This syndrome is linked to loss-of-function mutation in gene *SPINK-5* which encodes for LEKTI (lymphoepithelial Kazal type inhibitor), which is also a protease inhibitor (14,63,64).

INFLAMMATION AND THE ROLE OF IMMUNE SYSTEM

Inflammation is another independent factor that reduces levels of the FLG and NMF (12). This occurs independently of *FLG* *mut* status (65,66). Inflammation results from increased SPs activity, which activates the primary cytokines, IL-1 α , and IL-1 β from their pro-forms stored in KCs (20). Increased protease activity is the result of elevated pH, and this is typically the first step in a cascade of inflammation (65-68). The second step is sustained antigen ingress through the defective barrier, leading to Th2-dominant infiltrate (11). Certain antimicrobial antigens are preferentially associated with AD and show a propensity to trigger AD, especially in FLG-deficient patients (21). These microbial antigens activate SP activity and further damage the barrier (more details in the section on antimicrobial function).

Despite current accumulating evidence on barrier-initiated pathogenesis of AD, there is evidence on other mechanisms whereby Th2 generated cytokines worsen AD (11,20,65,70). The key Th2 cytokines, IL-4, and IL-13 cause further aggravation of AD by causing the following negative effects: IL-4 causes inhibition of ceramide synthesis, inhibition of KC-differentia-

tion-linked proteins (loricrin and FLG), and decreased desmoglein-3 expression with compromised SC integrity (65,70-72).

The skin is immunologically active and the skin barrier has an immunological function. One of the key elements in the immunological barrier are PRRs. They can be transmembrane/intracellular receptors and include a group of toll-like receptors (TLR), through which KCs sense microbial invasion and injury (13,14,73). Activation of TLRs on KCs can upregulate proinflammatory cytokine expression via the activation of NF- κ B (73). Cells secrete cytokines, such as TNF- α , and interleukins such as IL-6 and IL-1 α , which results in stimulation of cell proliferation and lipid synthesis (13,14,73). It is interesting that acute barrier disruption causes an increase in cytokines crucial for barrier repair, but chronic disruption, on the other hand, leads to inflammation and epidermal proliferation (13,14).

Although the role of the innate immune system has not yet been elucidated, it is known that activation of innate immune receptors results in the release of various inflammatory mediators derived from KCs which probably decrease FLG expression (75,76). Therefore, inflammation causes decreased FLG expression, regardless of *FLG* mutation status.

One of the hallmarks of the extrinsic variant of AD is elevated serum IgE. Serum IgE acts as an autoantibody, but it is still not known whether it targets FLG as well; it is however known that IgE autoreacts with a variety of KC-antigens (77). Autoantigens are released from damaged tissue triggered by mechanical injury/scratching (78).

It recently was discovered that the gene MAP-17 is associated with the T-helper cell cytokine-induced down regulation of FLG transcription in human KCs (79). Various cytokines such as IL-4, IL-17, IL-22, IL-25, and IL-31 can upregulate the MAP-17 gene, which further down-regulates FLG synthesis, CER production, and cell-to-cell adhesion (80-84). This provides a link between altered FLG and lipids in patients with AD.

ROLE OF LIPIDS

The structure of the barrier is closely related to its function. FLG has an enormous impact on the barrier, but a second very important cause of barrier alteration is change in the structure and composition of SC lipids. The permeability barrier resides in the SC which is multilayered tissue composed of flattened anucleate corneocytes surrounded by multiple lamellae sheets enriched in ceramides (CER), cholesterol (CHOL), and free fatty acids (FFA) (15). Hydrophobic lipids within the extracellular domain have a significant

role because they inhibit the outward movement of the water (15). Lipids are delivered as their precursors through secretion of the epidermal lamellar body (LB). As the SC is formed, LB deliver lipid constituents, lipid precursors, and enzymes required to generate CER and FFAs that are required for their organization into mature membrane structures (15). Additionally, proteases and their inhibitors derive from LB and orchestrate the orderly digestion of corneodesmosomes, allowing corneocytes to shed invisibly at the skin surface (15,16,85). CER are the main components of the multilayered lamellar bilayers between the corneocytes and thus the key factor in water retention and overall integrity of the barrier (86). It is well known that CER, with CHOL and FFA in a ratio of 3:1:1, are the most prominent group of physiological lipids and show alterations in skin of patients with AD. Different classes of CER differ in the length of the free fatty acid chain and sphingosin base. To date, 12 CER subclasses have been identified in humans, showing a wide chain length distribution (87-89). CER play a crucial role in the lipid organization due to their characteristic molecular architecture (90). In healthy SC, lipids form two lamellar phases with repeat distances of 6 nm and 13 nm, called the short periodicity phase and long periodicity phase (91). Lamellar lipids have a dense orthorhombic lateral organization, although a subpopulation of lipids can be densely packed in a hexagonal organization (92,93). Several studies on patients with AD reported reduction in total ceramids and reduction in long-chain CER and FFA (94-97). This substitution of shorter chain FFA results in lipid disorganization that likely further compromises barrier function (87,98). The relative chain length of CER is shorter and this correlates with an altered lipid organization and barrier function. This effect is *FLG* *mut* independent, but is associated with the levels of NMF (87). This fact suggests that *FLG* (genotype), NMF (phenotype), and other (translational and environmental) factors can influence NMF levels (87). Copy number variation of *FLG* and interleukin levels can probably downregulate FLG expression (70). NMF levels lead to elevated pH levels which may affect enzymes involved in CER synthesis and proteolytic activity (87,99-101). Changes in lipid composition and organization are observed in lesional skin, but also in nonlesional skin where increased levels of extremely short C34 chains were found. It is also reported that CER chains length is an important determinant of lateral lipid organization (87,97). These changes in CER chain length distribution correlate with changes in lipid organization, skin barrier function, disease severity, and levels of NMF. Therefore, chain length of CER is an important factor in skin barrier dysfunction. It is

suggested that this increase in short chains is due to a misbalance in the activity of some of the members of the elongase family, since the elongase family plays an important role in the elongation of fatty acids in the epidermis (98). Some other studies showed that the CER composition and chain length, rather than the ratio between lipid classes, plays a major role in the increased transepidermal water loss (TEWL) in nonlesional skin (102).

According to some authors, FLG deficiency also affects the delivery of lipids (12). Due to cytoskeletal abnormalities, loading into LB is partially compromised, resulting in some KCs being deficient in LB (103). This could reduce the normal quantities and organization of the extracellular lamellar bilayers (104). This provides a link between FLG and lipid composition of the epidermis.

Th2 cytokines can also compromise barrier function by downregulating expression of multiple epidermal structural proteins, lipid synthetic enzymes, and antimicrobial peptides (68). All of these inherited and acquired abnormalities converge on the lamellar body secretory system, producing abnormalities in lipid composition, secretion, and/or extracellular lamellar membrane organization, as well as antimicrobial defense (68). It has been recently shown that mutations in the genes *FATP4* (encodes for FATP4- fatty acid transporter in the suprabasal layer of epidermis) and *Tmem79* (encodes for a component in the lamellar granule secretory system) both associate with an increased risk of AD. These are both independent of *FLG mut* and can emphasize the importance of altered lipid production and secretion in the pathogenesis of AD (12,105,106).

Inflammation is another significant factor that can influence lipid status (12). In the chronic phase of AD, γ -IFN down-regulates two fatty acid elongases (*ELOVL1* and *ELOVL4*) required to generate the very long chain N-acyl fatty acids in CER and FFA (98). In this way, inflammation negatively influences lipid composition.

pH OF THE BARRIER AND PROTEASE ACTIVITY

Maintenance of adequate pH is very important for adequate skin barrier and function by preventing unnecessary activation of serine proteases (85,99,101). As already mentioned, reduction of the epidermal FLG results in reduction of its NMF, which is responsible for maintaining acidic pH. There is an allele dose-dependent effect, and homozygous *FLG mut* carriers usually have the highest pH levels (50,51). There is a compensatory mechanism due to upregulation of

Na⁺/H⁺ antiporter (NHE-1) which seems to counteract this pH increase, which may explain why elevated pH is not always seen in all *FLG mut* carriers (107). pH-sensitive proteases are activated following the pH increase (12). This causes premature degradation of corneodesmosomes and compromises intercellular connections and activates IL-1 α and IL-1 β (108,109). The most compelling evidence comes from Netherton syndrome (12,108). Protease activation impairs CER production and likely stimulates Th2 inflammation in AD (12). In addition to the protease effect of kalikreins, they also bind to protease activated receptors type 2 (PARS2) which downregulate lamellar body secretion and initiate a cascade of innate inflammatory responses, including activation of thymic stromal lymphoprotein (TSLP) from KCs. TSLP is a master switch for initiation of Th2 inflammatory responses (12,108,110,111). TSLP is produced by KCs, and secretion is induced by exposure to allergens, microorganisms, or mechanical injury (111-113). TSLP has an important role in the etiopathogenesis of itch, since it induces cutaneous nerves to release neuropeptides causing itch, with the consequence of scratching and further damage to the barrier and secondary superinfection (114).

INCREASED ALLERGEN PENETRATION AND ENHANCED IMMUNE REACTIVITY

FLG deficiency permits increased allergen penetration and enhances immune reactivity (12). *FLG*-null mouse is a model for ichthyosis vulgaris without AD, and these mice show accelerated allergen penetration through the SC as well as enhanced contact hypersensitivity response (115,116). In such cases, double allele mutations in *FLG* cause inherited skin barrier dysfunction (106). Another piece of evidence comes from the "flaky-tail" mouse with matted mutation (*Tmem 79*) which shows spontaneous inflammation and also increased penetration of allergens as well as a low threshold to irritation to allergens and skin irritants (116). Allergic sensitization and inflammation causes further exacerbation of the barrier deficit, which can be observed in the elevation of TEWL. Exposure to allergens or other stressors activates the inflammasome, protease, and downstream immune mechanisms (12). Some microorganisms can secrete and activate serine proteases and cause proteolytic damage to the barrier, but also cause release of active forms of IL-1 α and IL-1 β from KCs and initiate inflammation (117,118). Additionally, in conditions of *FLG* deficiency, stimulation of TLR 3 enhances production of TSLP (119,120). These findings suggest a link between *FLG* deficiency, Th2 promotion, and systemic disease (12,120). These observations on mouse mod-

els are yet to be confirmed in humans, but results from metaanalysis done in 2009 show that patients with AD and with FLG mutations have increased risk of sensitization to allergens and development of asthma, allergic rhinitis, and food allergy (121).

ANTIMICROBIAL DEFENCE

Another important function of the skin barrier is the antimicrobial function, which strongly encourages the growth of nonpathogenic flora (122). Healthy skin is colonized by large numbers of microorganisms, which are classified into resident and transient flora (123). Resident flora colonizes the skin in proportionally constant numbers and includes coagulase-negative staphylococci (*S. epidermidis*, *S. hemolyticus*, *S. hominis*). Transient flora is caused by contact of the skin with external objects. *Staphylococcus aureus* (SA) is not a member of resident flora (123). It has prevalence of approximately 5% in healthy population (124). More than 90% of patients with AD have SA on their lesional, and 30-100% on nonlesional skin (125,126). Permeability and antimicrobial dysfunction are well-known features of AD and are co-regulated and interdependent (19,61,67,68,127). Failure of the permeability barrier favors secondary infection, and secondary infection further compromises the barrier (19). Again, FLG plays an important role (12). FLG seems to protect against colonization of certain microorganism (12). *Staphylococcus aureus* (SA) has an important role in etiopathogenesis of AD. The skin of patients with AD exhibits a striking susceptibility to colonization and infection with SA (123). Patients with AD have the highest counts of SA on lesional skin, but often colony counts are elevated on even nonlesional/clinically normal skin (128). There is an extreme difference between lesional and nonlesional skin, and these counts differ by 100 to 1000 times (123,129). Indeed, SA colonization is both a cause and a consequence of allergic skin inflammation (123). Impaired barrier in AD predisposes to pathogen colonization not only due to increased pH, but also because of reduced levels of FFA and ceramide metabolite sphingosine which exhibits potent antimicrobial activity (19, 130,131). Surface proteins on SA downregulate epidermal FFA production, therefore aggravating permeability and antimicrobial function (132). Adherence of SA is increased in patients with AD due to injury of the skin barrier which leads to exposure of extracellular matrix adhesins for SA, epidermal and dermal laminin, and fibronectin (133,134). Fibronectin in combination with plasma exudation of fibrinogen allows SA to bind to the skin (133,134). Scratching further damages the barrier and causes cytokine release with upregulation of the expression

of adhesins (135). There is evidence that Th2 inflammation, or, more precisely, IL-4 plays important role in the enhancement of SA binding by inducing the production of fibronectin (134,136). Alteration of lipid composition is another contributing factor due to decrease in ceramides which leads to dry and cracked skin and predisposes to colonization (137). SA stimulates hydrolysis of CER by bacteria-produced ceramidases (138). Sphingosine exerts a potent antimicrobial effect in normal conditions, but, as a result of reduced activities of acid ceramidases and decreased levels of CER in general, the levels of sphingosine are also reduced (139). Normal FLG expression increases sphingomyelinase secretion (140).

Another important reason for SA colonization is defective innate immune response (122,123,132). There are 2 classes of endogenous antimicrobial peptides; β -defensins and cathelicidins, produced by KCs with antimicrobial effects against bacterial, fungal, and viral antigens (123). Antimicrobial peptides are delivered to SC intercellular domains via secretion of LB contents (19,141-143). Some of these antimicrobial peptides are constitutionally produced, while others (HBD-2, LL-37) are induced by TNF- α following skin inflammation or injury (123). These endogenous antimicrobial peptides are essential for defending the skin against bacterial infection (144). Members of two key families of antimicrobial proteins, the human cathelicidin product (hCAP), LL-37, and human β -defensins (hBD) 2 and 3 are downregulated in Th-2-dependent fashion (145-147). Fragments of hCAP and hBD3 exhibit potent antistaphylococcal activity (147).

In 70% of all patients with AD, non-toxin producing-SA strains are replaced with enterotoxin-producing strains and exacerbate AD via 3 mechanisms: these strains cause more frequent clinically apparent reactions; some toxins stimulate pruritus and production of specific IgE; and finally, some toxins act as superantigens by stimulating T- and B-cell production and switching to allergen-specific or superantigens that stimulate IgE production (19,66,122,123,148). Staphylococcal superantigens may directly stimulate antigen presenting cells and KCs to produce several proinflammatory cytokines, such as IL-1, TNF- α , and IL-12 and contribute to persistence and exacerbation of allergic skin inflammation (123,149). They recognize and stimulate T cells bearing the specific TCR β which results in massive activation of polyclonal T cells (up to 20% of total T cell population) (123). Superantigen-producing SA strains further exacerbate AD through augmentation of IgE production and development of specific IgE against SA exotoxins (122). It has been reported that T cells stimulated with superantigens become resistant to immunosuppressive effect of

corticosteroids (123,150). Activated T cells produce IL-31 which induces pruritus. It is well known fact that infections (such as folliculitis) are also extremely pruritic. Pruritus starts an itch-scratch cycle which causes further damages to the barrier and facilitates spreading of the infection (122). Another very interesting observation is that SA- α toxin preferentially destroys FLG-deficient KCs, so FLG again plays an important role (140). FLG breakdown products reduce growth rates of SA (151). In general, patients with AD with *FLG* *mut* have 7-fold increased risk of bacterial infection when compared to FLG wild type patients (12,152). Some microbes such as SA, dust mites, and scabies induce proteolytic activity with cleaving corneodesmosomal proteins and FLG, contributing further to the cycle of inflammation and pruritus.

Patients with AD are also prone to viral infections, especially to herpes simplex (7,153). Eczema herpeticum (EH) is clinically defined as disseminated herpes simplex viruses (HSV) infection which is almost exclusively seen in patients with AD (154). Nectin-1 is an entry receptor for HSV (155). Nectin-1, a cell adhesion molecule belonging to the immunoglobulin superfamily, can bind to virion glycoprotein D (gD) to mediate entry of HSV. Nectin-1 colocalizes with E-cadherin at adherens junctions in epithelial cells. The disruption of cell junctions can result in the redistribution of nectin-1 (155). Lack of plasmacytoid dendritic cells, cathelicidin production, and an abnormal IFN- γ response to HSV all contribute to pathogenesis of eczema herpeticum (156-158). The cathelicidin peptide LL-37 possesses antiviral activity against HSV and demonstrates the importance of variable skin expression of cathelicidins in controlling susceptibility to eczema herpeticum (156). Additionally, serum IgE levels might be a surrogate marker for innate immune function and serve as a biomarker for determining which patients with AD are susceptible eczema herpeticum (156).

Hinz *et al.* showed that patients with AD, recurrent HSV infection, and a history of eczema herpeticum had higher serum IgE levels, more severe AD, and a higher IgE sensitization profile when compared to a group without history of eczema herpeticum (159). It is interesting to point out that staphylococcal α -toxin increases the HSV load and therefore contributes to EH (160). A subset of patients with AD is prone to disseminated herpes simplex virus (HSV) infection, and biomarkers that identify this subgroup are lacking. A recent study has found that the two master regulators of innate immune responses IRF3 and IRF7 are suppressed in this subgroup, suggesting that abnormalities of up-stream of IRF3 and IRF7 signal pathways (161).

CONCLUSIONS

FLG has the central role in the etiopathogenesis of AD, since FLG deficiency affects epidermal protein expression and organization. A loss of function mutation in *FLG* is strongly associated with an elevated pH of the SC surface which might negatively influence SC-lipid production, activity of various proteases involved in inflammatory processes, and desquamation and antimicrobial defense. Not all patients can attribute their AD to FLG mutations. Up to 40% of patients with AD have one or two FLG mutations, and these mutations are associated with early onset as well as more severe and prolonged disease. Other patients with no FLG mutations have other causes of impaired barrier. Lipids are another very important determinant of skin barrier. Altered composition and structure of the lipids has a great impact on the function of the barrier. Since many proteolytic enzymes and protease inhibitors are involved in obtaining the normal function and structure of the barrier, there are numerous factors which can be influenced and whose function can be altered. All important functions of the barrier are a result of the barrier's structure and organization. Except genetic predisposition, where FLG mutation has the greatest influence, a many exogenous and endogenous stressors can further compromise the barrier. All traditional, "gold standard" therapeutic options today aim at inflammation and modulation of inflammatory response. We now know that everyday skin care and use of emollients is extremely important. What kind of emollients patients use is also very important, since every local therapy has an impact on the barrier. Since the barrier is impaired in patients with AD, some local products can even worsen and further compromise the barrier. Understanding the structure of the barrier and complexity of the function is a first step in understanding the etiopathogenesis of AD. It seems that every patient has AD with their own, unique set of mutations of important structural proteins, lipids, and proteolytic enzymes and their inhibitors, so it is probably wise to determine what kind of impairment of the barrier patient has and use this information in the therapeutic approach. It would be also advisable to take this into consideration in everyday life beginning with birth, since most of authors today the barrier impairment to be the first step in etiopathogenesis, with numerous genetic and environmental impacts. It is likely that in future we will apply the lessons from the barrier to create a more complex therapeutic approach. Corticosteroids and immunomodulators have an impact only on immunological response, but do not influence the barrier impairment. The barrier is also influenced by psychosociological stress and numer-

ous exogenous stressors that are common in modern life, such as environmental pollution and numerous hygiene products which cause further damage to the barrier. The rational approach would be aiming at reduction of consequences caused by genetics, such as FLG mutation and FLG deficiency, since epidermal FLG level is not only determined by the mutation itself. Except the genetic influence, another important determinant are numerous endogenous and exogenous stressors which further diminish barrier function. Local therapy can help in breaking the scratch-itch cycle and inflammation, since scratching causes mechanical damage to the barrier with the consequence of inflammatory response driven by damaged keratinocytes. In patients who show lipid aberration, a wise approach would be aiming at normalizing ceramide chain length distribution. Recently, much effort has been made to understand the role of the emollients, since their composition and characteristics are a very important determinant that can help improve impairment of the barrier.

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